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Patentanmeldung Nr. Patent application No. Demande de brevet n°

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
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If no title is shown please refer to the description.
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Compounds binding to P-selectin

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Title: Compounds binding to P-selectin

DESCRIPTION

FIELD OF THE INVENTION

The present invention relates to compounds which bind selectively to the adhesion molecule human P-selectin, to methods for preparing such compounds, to the use of such compounds in therapeutic or diagnostic methods and in pharmaceutical compositions, to nucleic acids encoding for proteinaceous materials comprising the amino acid sequences of said compounds, to gene delivery vehicles comprising such nucleic acids, to binding molecules binding to said compounds, and to a method for determining whether a compound is capable of binding to P-selectin.

BACKGROUND OF THE INVENTION

In recent years, cell surface adhesion molecules have become recognized as key mediators in numerous cellular processes including cell growth, differentiation, immune cell transmigration and response, and cancer metastasis.

Four major categories of adhesion molecules have been identified: the immunoglobulin superfamily cell adhesion molecules (CAMs), cadherins, integrins, and selectins.

The selectins represent a family of presently three transmembraneous, carbohydrate-binding glycoproteins: "endothelial" E-selectin, "leukocyte" L-selectin, and "platelet" P-selectin. All three selectins are divalent cation (e.g. calcium) dependent and possess an extracellular domain

with a carbohydrate recognition motif, an epidermal growth factor-like motif, and some smaller domains related to complement-regulatory proteins.

Human P-selectin (also referred to as GMP-140, LECAM-3, PADGEM, CD62, and CD62P) is expressed by platelets and endothelial cells. When expressed on the surfaces of these cells, its most notable effect is the slowing of leukocytes as these leave the capillaries and enter the postcapillary venules, the latter representing the major site of leukocyte-endothelium adhesion. The slowing process is observed as leukocyte rolling, signifying an initial adhesion with relatively low affinity. The firm adhesion of rolling leukocytes is primarily mediated by integrins.

In endothelial cells, P-selectin is stored on Weibel-Palade bodies; in platelets, it is found in the α-granules. Following activation, P-selectin is mobilized to the cell surfaces within a few minutes in response to a variety of inflammatory or thrombogenic agents. The endothelial P-selectin's primarily function is to recruit leukocytes into postcapillary venules, while platelet P-selectin also results in the formation of thrombi. One of the presently known natural ligands of P-selectin is PSGL-1 (P-selectin glycoprotein ligand-1), a 160 kDa sialoprotein expressed on the surface of leukocytes where it is concentrated at the uropod. More detailed descriptions of the structure and functions of P-selectin are found in numerous publications, such as J. Panes, Pathophysiology 5, 271 (1999); F. Chamoun *et al.*, Frontiers in Bioscience 5, e103 (Nov. 1, 2000); S.-I. Hayachi, Circulation 102, 1710 (2000).

Inflammation and inflammatory processes play a major role in the pathophysiology of numerous diseases and conditions. Conditions of the brain in which increased selectin levels were found, and which may therefore involve selectin-mediated pathophysiological events, include severe traumatic brain injury, relapsing-remitting multiple sclerosis, cerebral artery occlusion, ischemia, and stroke. Conditions of the heart in which selectins are suggested to play a role include acute myocardial infarct, arterial injury, such as produced by angioplasty, and ischemia. Similarly, selectins are involved in

conditions of the kidneys, such as renal injury from ischemia and reperfusion, and renal failure. Furthermore, selectins appear to play a role in organ transplant rejection, cold ischemia, hemorrhagic shock, septic shock, tumor metastasis, chronic inflammation, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, restenosis, angiogenesis, disseminated intravascular coagulation, adult respiratory stress syndrome, and circulatory shock.

Thus, it would seem feasible to improve these and other conditions involving the activation of endothelial cells and leukocytes and specifically the mobilization and expression of P-selectin by specifically interrupting the P-selectin cascades. This can be done, for instance, by the administration of ligands which selectively bind to human P-selectin, but which do not possess its bioactivity. By this method, mobilized P-selectin could be inactivated and leukocyte-induced tissue damage prevented. Potentially, the same effect could be achieved by gene therapy, provided the P-selectin ligand or antagonist is a peptide or modified peptide. According to this method, somatic cells of a person in need of the therapy would be transfected with an expression vector carrying a DNA sequence encoding a P-selectin antagonist.

P-selectin ligands or antagonists may also be used for the prevention of diseases and conditions described above. Furthermore, such ligands may also be useful in the *in vivo* or *in vitro* diagnosis of these diseases.

Various attempts have been made in recent years to identify or create such selective ligands to P-selectin. So far, a number of substances were tested, but no clinical studies have yet provided conclusive evidence that any of these compounds produce the desired clinical effects while being tolerable in terms of side effects.

For instance, antibodies to P-selectin that were produced and tested in animal models, were found to protect kidneys from ischemic-reperfusion injury (H. C. Rabb *et al.*, JASN 5, 907, 1997; US-A-6,033,667). In another study, a recombinant soluble form of P-selectin glycoprotein ligand-1 (rPSGL-Ig) was used to inhibit thrombosis in cats (M. J. Eppihimer *et al.*,

Arteriosclerosis, Thrombosis, and Vascular Biology 20, 2483, 2000). WO-A-96/09309 discloses oligosaccharide structures that are ligands to E- and P-selectin. WO-A-99/41363 discloses podocalyxin-like proteins that bind to selectins. WO-A-00/41711 describes various smaller peptides or peptide sequences that bind to members of the human selectin family; most of the sequences comprise one or more units of leucine or isoleucine.

As another approach to inhibit the P-selectin cascade, various peptides derived from the lectin domain of the selectin family were found to inhibit neutrophil adhesion to P-selectin (e. g. US-A-6,111,065 and US-A-5,916,876); these peptides probably bind to P-selectin receptors on leukocytes.

In WO-A-94/05269, peptides are described which inhibit binding of selectings such as P-selectin, E-selectin and L-selectin. These peptides have as their core region portions of the 11-18 amino acid sequence of P-selectin, E-selectin or L-selectin. Further, WO-A-95/31210 relates to peptides and compounds that bind selectins including endothelium leukocyte adhesion molecule 1 (ELAM-1). These peptides are used for blocking adhesion of leukocytes to the selectins, *i.e.* especially E-selectin, but also P-selectin or L-selectin, for the purpose of inhibiting inflammation.

Despite these efforts, there is still a need for substances with selective affinity to P-selectin, which can be used for preparing pharmaceutical compositions for the diagnosis, prevention and treatment of various diseases and conditions involving the adherence of leukocytes to vascular endothelial cells or to platelets. There is also a need for P-selectin ligands, which can be used as targeting molecules or moieties in pharmaceutical compositions for the targeting of drugs or genetic material to tissues expressing P-selectin.

OBJECTS OF THE INVENTION

It is therefore an object of the invention to provide compounds with affinity to human P-selectin.

In particular, it is an object of the invention to provide compounds which act as antagonists or partial antagonists of P-selectin.

It is another object of the invention to provide compounds which act as targeting ligands with an ability to target drugs and genetic material to cells and tissues expressing P-selectin.

A further object of the invention is the provision of methods for preparing such compounds.

Yet another object is the presentation of uses of such compounds, and of compositions which contain the compounds.

Other objects of the present invention will become clear on the basis of the following description.

SUMMARY OF THE INVENTION

In the copending EP application No. 01203314.8, and applications claiming priority thereof, compounds which comprise the peptidic sequence $XA_xA_3A_1A_2A_1Y$ and have selective affinity to human P-selectin have been disclosed. In this formule

A_1 is a D- or L-cysteine (C), D- or L-methionine (M), D- or L-valine (V) or an analogue thereof;

A_2 is a D- or L- aspartic acid (D) or an analogue thereof;

A_3 is a D- or L- phenylalanine (F), D- or L-tyrosine (Y) or D- or L-tryptophan (W) or an analogue thereof;

A_x is a D- or L-amino acid;

X marks the N-terminal side of said sequence and Y marks the C-terminal side of said sequence or X and Y together can form a cyclic system.

The present invention provides novel compounds with selective affinity to human P-selectin. The compounds of the invention are peptides or functional equivalents, such as modified peptides, peptide analogues, or peptidomimetics. They comprise the sequence $X(A_x)_mA_3A_1A_2A_1Y$, wherein A_1 is

a D- or L-amino acid selected from the group consisting of cysteine (C) and valine (V), or an analogue or mimetic thereof; A_2 is a D- or L- aspartic acid (D) or an analogue or mimetic thereof; and A_3 is a D- or L-amino acid selected from the group consisting of phenylalanine (F) and tryptophan (W), or an analogue or mimetic thereof; A_x is any D- or L-amino acid selected from the group consisting of glutamic acid, aspartic acid, glycine, cysteine and analogues or mimetics thereof; X is a N-terminal group or sequence; Y is a C-terminal group or sequence. Either X or Y, or both X and Y, are substituted with the group R^1-$(Z)_n$-. Z is selected from -CO-, -O-, -NR^2-, and -CO-NR^2-. R^1 and R^2 are independently selected from H, a C_1-C_8 alkyl group, a C_2-C_8 alkyl group in which at least one hydrogen substituted C-atom is replaced with a hydrogen substituted N-, oxygen- or sulphur atom, a C_6-C_{14} aryl group which may be substituted with at least one group selected from the group consisting of a halogen atom, C_1-C_6-alkyl, -CF_3, -OH, -O-C_1-C_6-alkyl, -COOH, -COO-C_1-C_6-alkyl, -NO_2, -NH_2, -NH-C_1-C_6-alkyl, -N-(C_1-C_6-alkyl)$_2$ and -SO_3H, a heteroaryl group which is selected from 5- or 6-membered ring systems and benzo-condensed ring systems, and has at least one heteroatom selected from the group consisting of nitrogen, oxygen and sulphur, wherein said heteroaryl group may be substituted with at least one group selected from the group consisting of a halogen, -C_1-C_6-alkyl, -CF_3, -OH, -O-C_1-C_6-alkyl, -COOH, -COO-C_1-C_6-alkyl, -NO_2, -NH_2, -NH-C_1-C_6-alkyl, -N-(C_1-C_6-alkyl)$_2$ and -SO_3H, or an aralkyl group comprising an alkyl group as defined above and an aryl group or heteroaryl group as defined above. The indices m and n represent integers independently selected from 0 and 1. However, n is not 0 when R^1 is H. The scompounds have a high affinity to human P-selectin in the low micromolar and even nanomolar range.

The compounds of the present invention differ from the compounds described in said copending application in that the N-terminus, C-terminus, or when the N- and C-terminus are linked together, the combined N + C terminus are modified by a group R'-(Z)n. This modification is on a primary amine

group. It was found that the modified compounds of the present invention are potent inhibitors of P-selectin binding. The compounds of the invention are peptides having low micromolar to low nanomolar affinity towards P-selectin, which make them about equally potent as the natural ligand P-selectin glycoprotein ligand-1. The compounds can for instance be used in atherothrombotic therapy.

The compound EWVDV described in the above-mentioned copending application has an affinity for P-selectin of about (IC_{50}=) 2 μm. Its tetramer on streptavidin has an IC_{50} of about 2 μm. For *in vivo* use it would be highly desirable to have relatively simple peptides with a very low affinity for P-selectin. Such peptides are the peptides of the present invention.

Instead of using conventional replacement of individual amino acids by naturally occurring amino acids, the compounds of said copending application were modified reactions and particularly acylation on available amine groups.

It was found that N-terminally modified compounds of the invention were more effective (potent and/or specific) than the corresponding C-terminally modified compounds containing the same modifications. Very good results were obtained with modification by 1,3,5-tricarboxylic acid or gallic acid.

In a second aspect, the invention provides methods for the preparation of such compounds. The methods include the chemical and/or enzymatic ligation of amino acids monomers or oligomers to assemble the compounds. They also include the expression of nucleic acid sequences encoding the compounds in host cells, using a vector for transfecting the host cells with the nucleic acid sequences.

The compounds obtained – as said – modified by reaction with a suitable $R^1(Z)_n$- source, preferably a $R^1(Z)_n$- OH group. Said source reacts with a primary amine group in the peptide compound.

When the compounds of said copending application are made by standard Fmoc chemistry and the protecting Fmoc group is removed, the N-terminus is immediately available for the reaction introducing the $R^1(Z)_n$-group.

When the C-terminus has no free amine available, the said source can be reacted with an added lysine, which lysine can suitably be added as its DDE derivative.

(DDE is 4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-ethyl; it can selectively be removed with 2% hydrazine without affecting acid-labile side chain protecting groups).

In a further aspect, the invention relates to the use of the compounds of the invention for preparing pharmaceutical or diagnostic compositions which are suitable for inhibiting the binding of leukocytes to platelets and/or endothelial cells *in vitro* and *in vivo*. As medicaments, the compositions may be useful in the treatment and prevention of conditions and disorders which involve the activation of P-selectin-mediated binding of leukocytes to platelets and/or endothelial cells, such as thrombotic disorders, ischemia, restenosis, atherosclerosis, renal failure, parasitic diseases, tumors and tumor metastases. Pharmaceutical compositions containing the compounds of the invention may be adapted for various routes of administration, such as parenteral, oral, transmucosal, nasal, or pulmonary. They may further contain drug targeting agents, bioavailability enhancement agents, or active ingredients other than compounds of the invention, and provide for immediate or modified release.

In yet a further aspect, the present invention relates to a method for determining whether a molecule comprises a binding affinity for P-selectin, comprising contacting of P-selectin or a functional equivalent thereof with said molecule and with a compound according to the invention, followed by determining whether binding of said compound to said P-selectin or functional analogue thereof is reduced.

Further, the invention relates to a nucleic acid encoding a proteinaceous molecule comprising an amino acid sequence $X(A_x)_mA_3A_1A_2A_1Y$, wherein A_x, A_3, A_1, A_2, and m are as defined herein-above and wherein X is the N-terminal side of said sequence and Y is the C-terminal side of said sequence or a functional equivalent thereof, as defined above. The said nucleic acid can be used for the preparation of a medicament, while in addition gene delivery vehicles comprising said nucleic acid are also within the scope of the present invention.

In a further aspect, the present invention also encompasses binding molecules which are capable of specifically binding a compound of the invention.

Furthermore, the present invention relates to a method for determining whether a compound is capable of binding to human P-selectin, comprising substituting in a compound according to the invention, an amino acid for a conservative amino acid and determining whether the resulting compound is capable of binding to said P-selectin.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the invention have an affinity to human P-selectin, a membrane glycoprotein expressed by vascular endothelial cell and platelets, which is involved in leukocyte adhesion to the endothelium and platelets. The affinity or binding characteristics of compounds to P-selectin can be quantified, for example, in terms of the affinity constant (IC_{50}). Typically, an affinity constant of about 50-100 μM or less would be considered as evidence for affinity and binding. More desirable for ligands are substances with affinity constants of about 10 μM or less. The highest affinity constants attainable for the non-covalent type bonds playing a role in the interactions or bindings in accordance with the present invention is about 10^{-15} M. Generally,

however, the affinity constants are higher than about 10^{-12} M and in most cases higher than about 10^{-9} M.

Furthermore, a compound of the invention comprises a peptide or a molecular structure that is related to a peptide, herein referred to as functional equivalent.

Peptides are defined as amides that are derived from two or more amino acids by combination of the amino group of one acid with the carboxyl group of another (Merriam Webster Medical Dictionary ©2001). As used herein, a peptide may also refer to a peptidic structure within a molecule. Typically, peptides are composed of naturally occurring L-α-amino acids, which are alanine (Ala or A), arginine (Arg or R), asparagine (Asn or N), aspartic acid (Asp or D), cysteine (Cys or C), glutamine (Gln or Q), glutamic acid (Glu or E), glycine (Gly or G), histidine (His or H), isoleucine (Ile or I), leucine (Leu or L), lysine (Lys or K), methionine (Met or M), phenylalanine (Phe or F), proline (Pro or P), serine (Ser or S), threonine (Thr or T), tryptophan (Trp or W), tyrosine (Tyr or Y), and valine (Val or V).

Functional equivalents of the peptides of the invention are proteinaceous molecules, comprising the same human P-selectin binding activity in kind, but not necessarily in amount, and may, for instance, be modified peptides, peptoids, peptide analogues or peptidomimetics.

Modified peptides are molecules derived from peptides by the introduction of substituents or functional groups which are not present in naturally occurring amino acids. The term also includes compounds which are obtained by the reaction of peptides with molecules from other chemical categories, whether these molecules are naturally occurring or not. For instance, biotinylated peptides, glycoproteins, and lipoproteins are frequently found in nature, while peptides modified with polyethylene glycol, such as pegylated interferon α-2b (Peg-Intron®), are examples of chemically modified peptides that have been designed to alter some, but not all of the peptides' properties.

Peptoids, like peptides, are typically amides of two or more amino acids. However, they are frequently not directly derived from naturally occurring amino acids, but rather of various types of chemically synthesized L- or D-amino acids.

Peptidomimetics, in their broadest scope, are compounds which are in their functional structure more or less similar to a peptide, but which may also contain non-peptidic bonds in the backbone, or D-amino acids. In general, peptidomimetics serve as substitutes for native peptides in the interaction with receptors and enzymes (Pharmaceutical Biotechnology, Ed. D. J. A. Crommelin and R. D. Sindelar, Harwood Academic Publishers, 1997, p. 138). Pseudopeptides, a class of peptidomimetics, are compounds containing amide bond isosteres instead of amide bonds (*ibid.*, pp. 137-140).

Compounds of the invention also include salts of peptides or functional equivalents, such as pharmaceutically acceptable acid- or base addition salts. They also include multimers of peptides or functional equivalents.

The compounds of the invention are characterized in that they comprise the sequence $X(A_x)_mA_3A_1A_2A_1Y$, wherein A_1 is a D- or L-cysteine (C), or D- or L-valine (V) or an analogue thereof; A_2 is a D- or L- aspartic acid (D) or an analogue thereof; A_3 is a D- or L- phenylalanine (F), or D- or L-tryptophan (W) or an analogue thereof; A_x is a D- or L-amino acid selected from the group consisting of glutamic acid (E), aspartic acid (D), glycine (G) , cysteine (C) and analogues thereof; X marks the N-terminal side of said sequence and Y marks the C-terminal side of said sequence, where X and Y together may form a cyclic system, m is an integer of 0 or 1. X is a hydrogen or a residue comprising 1 to 6 D- or L-amino acids; Y is a hydroxyl or a residue comprising 1 to 11 D- or L-amino acids terminated by a hydroxyl. Furthermore, at least one of X and Y, or both, is substituted with a substituent R^1-$(Z)_n$-, wherein n is an integer of 0 or 1. If both X and Y are substituted, the respective substituents are selected independently.

According to the invention, the two units of A_1 within the sequence are selected independently; they can be identical or different from each other. Preferably, at least one of the A_1 units represents valine (V). More preferably, both A_1 units are valine (V). In another embodiment, A_1 is an analogue or mimetic of cysteine (C), methionine (M), or valine (V). Techniques for selecting appropriate analogues or mimetics of naturally occurring amino acids are well-known to those skilled in the art. For example, the pseudopeptide approach aims at achieving a higher chemical or enzymatic stability of a peptide while retaining its bioactivity. Peptide bonds which undergo rapid degradation *in vivo* are replaced by other functional groups to create bioisosteres or amide bond surrogates. Examples of bioisosteric groups are N-methyl amide, thioester, thioamide, ketomethylene, methyleneamino, retro-inverse amide, methylenethio, and methyleneoxy groups (*ibid.*, pp. 138-139). An approach to improve the selectivity of a peptide is to introduce conformational constraints to the backbone, which decreases the number of potential receptor or enzyme interactions. The constraints are most often achieved by the introduction of carbon-carbon double bonds (olefinic analogues) and ring structures (*ibid.*). A further method to identify analogues which mimic the functional structure of an amino acid such as cysteine (C), methionine (M), or valine (V) is computer-aided modeling. Preferred mimetic structures possess a side chain with similar charge or electronegativity, hydrophobicity and spatial orientation to said amino acids. In another embodiment, one or both A_1 units are D-amino acid analogues of cysteine (C), methionine (M), or valine (V).

As said, the present invention also encompasses functional equivalents of the said peptides. Equivalents can, among others, be found by amino acid substitution using conservative amino acid changes. Particularly, the invention also encompasses a method for determining whether a compound is capable of binding to human P-selectin, comprising substituting in a compound according to the invention, an amino acid for a conservative amino acid and determining whether the resulting compound is capable of binding to

said P-selectin. According to the invention, A_2 is a D- or L-aspartic acid with a solvent exposed side chain bearing a negative charge at physiological pH, or an analogue or mimetic thereof. For identifying appropriate analogues, the same principles apply as those that have been set forth with regard to A_1.

A_3 is a D- or L-amino acid with an aromatic side chain selected from the group consisting of phenylalanine (F), and tryptophan (W), or an analogue or mimetic thereof. More preferably, A_3 is an L-amino acid selected from this group. Most preferred is tryptophan (W). In other embodiments, A_3 is an analogue structure related to phenylalanine (F), or tryptophan (W) as defined above.

A_x is a D- or L-amino acid, as defined above, or an analogue or mimetic thereof. In one of the preferred embodiments, A_x is a D- or L- glutamic acid (E), or an analogue or mimetic thereof.

It has to be noted that the 2 units of A_1 are selected independently, *i. e.* they can be identical or different from each other.

X is defined as any N-terminal group or sequence. For example, X can simply be a hydrogen. In another embodiment, X is a sequence of up to 6 amino acids, or analogues or mimetics thereof. The person skilled in the art is competent to find suitable amino acids in this respect. The type of amino acids(s) should of course not affect e.g. by changing the configuration of the peptide or by changing the spatial orientation of the terminal carboxylic acid with respect to the core peptide the recognition by P-selectin in such a way that, the aimed effect is no longer obtained. Suitable analogues include amino substituted carboxylic acids, e.g. aminocyclohexanoic acid amino benzoic acid and amino caproic acid.

Y is defined as any C-terminal group or sequence. If no amino acids or analogues are comprised in Y, the C-terminal group may be a hydroxyl group. In a preferred embodiment, Y is a residue comprising 1 to 11 amino acids carrying a terminal hydroxyl group. The recognition by P-selectin is more tolerant to substituents at the C-terminus. The skilled person is competent to

find suitable amino acids in this respect. It is also preferred that at least one amino acid with a negatively charged side chain is present in Y, preferably being separated from the remaining $X(A_x)_mA_3A_1A_2A_1$ sequence by one to three amino acids. In a particularly preferred embodiment of the invention Y is D- or L- lysine (K), or comprises D- or L- lysine (K).

The compound of the invention can possess a cyclic or otherwise constrained backbone. In that case, X+Y together may suitably be linked through a cys-cys binding, although, of course, also other types of (chemical) linkages can be present, such as an amide binding, a thioether binding, a carbamate binding or an ester binding.

The length of the group X+Y is not particularly critical as long as the core sequence is recognised by P-selectin. Generally, the minimum length of X+Y corresponds with the length of 5-6 amino acids.

The group R^1-$(Z)_n$- with which X or Y, or both X and Y independently, are substituted, may be selected from R-CO groups wherein R is an alkyl, aryl or heteroaryl group. The alkyl group preferably has 2 to 8 carbon atoms, at least one of which may be replaced by a nitrogen, oxygen, or sulphur moiety. An aryl group has preferably 6 to 14 carbon atoms, and the group may be substituted with at least one group selected from the group consisting of OH, COOH, NO_2, NH_2, SO_3H and CF_3. Also substitutions on this group selected from –NHR, -CO(NHR), -CN(NHR), $-N_3$, $-(CH_2)_p$-OH, and $-(CH_2)_p$-COOH are suitable, wherein R is as defined hereinabove, while p is an integer of 1-8. The heteroaryl group has the same definition as the aryl group with the exception that at least one heteroatom selected from the group consisting of nitrogen, oxygen and sulphur is incorporated instead of a carbon atom. Preferably, the heteroaryl group is based on a .5- or 6-membered ring system or benzo-condensed ring system. Especially preferred acyl groups are 3,5-dicarboxyphenylcarbonyl and 3,4,5-trihydroxyphenylcarbonyl groups.

Further, also modifications based on tannic acid, caffeinic acid, oligohydroxyl aryl groups and oligocarboxy-aryl groups as well as derivatives thereof are preferred, especially on group X.

It is assumed that the R_1-$(Z)_n$ modification confers additional interaction of the peptide compound with the P-selectin binding pocket on P-selection, possibly by electrostatic interaction or H-bridge formation. This leads to a grain inaffinity for P-selectin.

Very good results are obtained for those compounds having an N-terminal amide function. Also very good results are obtained when using a linker between the N-terminal substituent group and amino acid A_x or A_3 in the amino acid sequence of the compounds of the invention. Suitable spacers are glycine and aminobutyric acid.

Compounds of the invention possess linear, branched, cyclic, or constrained backbones. For instance, peptides or functional equivalents with at least two cysteine (C) units can be cyclized by oxidation. If a compound is cyclized via such a disulfide bond, it is preferred that the participating cysteine units are members of X and Y, respectively. Cyclic structures are, in fact, an example for conformationally constrained backbones. Other types of constrained structures may also be introduced to decrease the conformational flexibility of the compound. Especially the presence of olefinic bonds or small ring structures in the backbone serve this purpose. Examples of such constraints are given in Pharmaceutical Biotechnology, Ed. D. J. A. Crommelin and R. D. Sindelar, Harwood Academic Publishers, 1997, p. 139f.

In one of the preferred embodiments, the compounds of the invention are provided as multimers of peptides or functional equivalents. As used herein, multimers, which in peptide chemistry are also called oligomers, refer to peptides, proteins, peptoids, peptidomimetics, or analogues thereof, which are composed of more than one peptide chain. For example, tetramers of biotinylated peptides comprising the sequence $X(A_x)_mA_3A_1A_2A_1Y$ were found to possess a substantially higher affinity to P-selectin than the peptides they

were composed of. Preferred multimers of the invention have an affinity constant for P-selectin of below 1/20, and especially below 1/100 of the affinity constant of the corresponding peptides.

In another type of multimer that can be created to form the compounds of the invention, single peptide or peptoid chains are coupled to a biocompatible protein, such as human serum albumin, humanized antibodies, liposomes, micelles, synthetic polymers, nanoparticles, and phages. Multimers can also represent peptide sequences which are serially coupled to each other via spacers, i.e. concatamers, or dendrimers, or clusters.

The compounds can generally be prepared by the methods that are known for the preparation of peptides and similar substances. Smaller compounds containing only a few amino acids or similar units, and preferably not more than 30-50 units, can be prepared by chemical and/or enzymatic ligation techniques, either using the classical approach in which the reactions take place in solution or suspension, or by employing the more modern solid phase approach, in which the peptide is assembled while being anchored to a solid surface, such as a polymeric bead. Larger compounds are typically synthesized by automatic solid phase peptide synthesizers.

Alternatively, the compounds can be prepared by known genetic engineering techniques. This approach is especially valid if the compound is indeed a peptide or a slightly modified peptide. For instance, a DNA sequence which encodes the compound can be associated or combined with an expression vector capable of transfecting cells. In another step of the method, host cells or target cells are transfected with said DNA by contacting the cells with the vector and the vector-associated DNA under conditions which allow transfection. In a further step, the host or target cells are cultured under conditions which allow the expression of the compound. Subsequently, the compound can be isolated. If the compound itself cannot be encoded or expressed but is very similar to a peptide that can be encoded or expressed, the method can be applied to prepare the peptide to which the compound is

similar, followed by one or more steps in which the peptide is modified by chemical or enzymatic techniques to prepare the compound.

Various types of vectors are suitable for this purpose, such as viral vectors, lipoplexes, polyplexes, microspheres, nanospheres, dendrimers, naked DNA, peptide delivery systems, lipids, especially cationic lipids, or liposomes made thereof, polymeric vectors, especially those made of polycationic polymers. Among the preferred viral vectors are retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, and virosomes. Preferred non-viral vectors include chitosan, SPLP, polymeric systems based on PLGA, polyethyleneimines, polylysines, polyphosphoamidates, poly(meth)acrylates, polyphosphazenes, DOPE, DOTAP, and DOTMA.

Some more comprehensive summaries of methods which can be applied in the preparation of the compounds are described in: W. F. Anderson, Nature 392 Supp., 30 April 1998, p. 25-30; Pharmaceutical Biotechnology, Ed. D. J. A. Crommelin and R. D. Sindelar, Harwood Academic Publishers, 1997, p. 53-70, 167-180, 123-152, 8-20; Protein Synthesis: Methods and Protocols, Ed. R. Martin, Humana Press, 1998, p. 1-442; Solid-Phase Peptide Synthesis, Ed. G. B. Fields, Academic Press, 1997, p. 1-780; Amino Acid and Peptide Synthesis, Oxford University Press, 1997, p. 1-89.

Salts of peptides or functional equivalents are prepared by known methods, which typically involve the mixing of the peptide or peptoid with either a pharmaceutically acceptable acid to form an acid addition salt, or with a pharmaceutically acceptable base to form a base addition salt. Whether an acid or a base is pharmaceutically acceptable can be easily decided by a person skilled in the art after taking the specific intended use of the compound into consideration. For instance, not all acids and bases that are acceptable for *in vitro* diagnostic compositions can be used for therapeutic compositions. Depending on the intended use, pharmaceutically acceptable acids include organic and inorganic acids such as formic acid, acetic acid, propionic acid, lactic acid, glycolic acid, oxalic acid, pyruvic acid, succinic acid, maleic acid,

malonic acid, cinnamic acid, sulfuric acid, hydrochloric acid, hydrobromic acid, nitric acid, perchloric acid, phosphoric acid, and thiocyanic acid, which form ammonium salts with free amino groups of peptides and functional equivalents. Pharmaceutically acceptable bases, which form carboxylate salts with free carboxylic groups of peptides and functional equivalents, include ethylamine, methylamine, dimethylamine, triethylamine, isopropylamine, diisopropylamine, and other mono-, di- and trialkylamines, as well as arylamines. Moreover, also pharmaceutically acceptable solvates are encompassed.

Multimers can, for example, be prepared by biotinylating the N-terminus of peptide or peptoid chains and subsequent complexation with streptavidin. As streptavidin is able to bind 4 biotin molecules or conjugates with high affinity, very stable tetrameric peptide complexes can be formed by this method. Multimers may be composed of identical or different peptides or functional equivalents. Preferably, however, the multimers of the invention are composed of two or more identical peptides or functional equivalents.

A further aspect of the invention refers to the uses of the disclosed compounds. Since the compounds bind selectively to P-selectin, they can, depending on their type of interaction with P-selectin after binding, function as antagonists, partial antagonists, or as mere targeting means to target conjugated substances to cells and tissues expressing P-selectin. Thus, the compounds can be advantageously used in pharmaceutical compositions. According to the invention, such pharmaceutical compositions are provided as well.

As used herein, the term "pharmaceutical composition" refers to therapeutic and diagnostic compositions, as well as to medicaments and diagnostics containing such compositions. Therapeutic compositions and medicaments are used for the prevention or treatment of diseases and other conditions of mammals of which conditions improvement is desirable.

Diagnostics and diagnostic compositions are used for the diagnosis of such diseases *in vivo* and *in vitro*.

A preferred use of the compounds is for preparing therapeutic compositions or medicaments to prevent or improve diseases and conditions involving the adhesion of leukocytes, such as monocytes and neutrophils, to the vascular endothelium and to platelets. The compounds can also be used in compositions for treating diseases in which the inhibition of P-selectin-mediated intracellular signaling is desirable.

For instance, compositions containing one or more compounds of the invention can contribute to controlling leukocyte-mediated inflammatory processes. It is known that activated leukocytes release toxic molecules which can damage normal tissue. These inflammatory responses, some of which also involve P-selectin-mediated platelet activation, are part of several pathological conditions, such as transplant rejection, cold ischemia, hemorrhagic shock, septic shock, tumor metastasis, chronic inflammation, rheumatoid arthritis, inflammatory bowel disease, atherosclerosis, restenosis, angiogenesis, disseminated intravascular coagulation, adult respiratory stress syndrome, circulatory shock, severe traumatic brain injury, relapsing-remitting multiple sclerosis, cerebral artery occlusion, ischemia, stroke, acute myocardial infarct, arterial injury, such as produced by angioplasty, myocardial ischemia, renal injury from ischemia and reperfusion, and renal failure.

In another embodiment, the compounds are used in the preparation of diagnostic compositions or products. Such compositions can be used for *in vitro* tests to quantify P-selectin concentrations in body fluids as markers for the diseases and conditions described above. They may be also used for *in vivo* diagnostic imaging procedures to monitor P-selectin mediated atherosclerosis, aneurisms, restenosis following percutaneous transluminal coronary angioplasty (post-PTCA restenosis), and other conditions selected from those in which P-selectin is mobilized. As an option for this use, a compound according to the invention may be conjugated with a chelator, which is

subsequently complexed with an isotropic label that is detectable by the chosen monitoring system.

Another use of the compounds is as that of a tool in research. For instance, they can be used to test the binding affinity of molecules to P-selectin or functional equivalents of P-selectin. To conduct this test method, P-selectin or a functional equivalent of P-selectin would be contacted and incubated with a molecule to be tested for binding affinity and with a compound of the invention. A reduced binding of the compound of the invention would indicate an affinity of the molecule to P-selectin.

The compounds can also be used as targeting molecules or conjugates in pharmaceutical compositions for the targeting of drugs or genetic material to tissues that express P-selectin. As conjugates, the compounds can be directly coupled with active molecules or nucleic acids that are to be delivered to such tissues. Alternatively, they can be incorporated into or anchored onto the surface of liposomes or other lipid vesicles, emulsion droplets, polymers, nano- or microparticles to obtain targeted vehicles for drugs or genetic material which is delivered to P-selectin expressing tissues.

The pharmaceutical compositions preferably contain one or more compounds with P-selectin affinity as disclosed herein and at least one carrier or excipient. As used herein, a carrier or excipient is any pharmaceutically acceptable substance or mixture of substances having no substantial pharmacological activity, which can be used as a vehicle or as an auxiliary substance to formulate a compound into dosage form which is stable and easy to administer. Examples of pharmaceutically acceptable excipients are found in the monographs of all major pharmacopoeias.

In one embodiment, the composition is formulated and processed for parenteral injection, preferably for intravascular injection, such as intravenous or intra-arterial, but also for intramuscular, subcutaneous, intralesional, intraperitoneal or other routes of parenteral administration. The same principles that govern the formulation of other drugs for these administration

routes will also teach those skilled in the arts on how to prepare such compositions. For instance, one of the requirements of parenteral dosage forms is their sterility. Other requirements are described in all major pharmacopoeias, such as in USP 24, in the monograph "General Requirements for Tests and Assays. 1. Injections", p. 1775-1777. To increase the stability of a parenteral formulation, it may be necessary to provide a dried dosage form which must be reconstituted before it can be administered. An example of such a dosage form is a freeze-dried or lyophilized formulation.

It may be desirable to administer a compound of the invention as a parenteral controlled release dosage form to avoid frequent injections and to improve the effectiveness and convenience of the therapy. Various methods of preparing such depot formulations are known. Prolonged release may be provided by solid implants, nanoparticles, nanocapsules, microparticles, microcapsules, emulsions, suspensions, oily solutions, liposomes, or similar structures.

Excipients that are particularly useful for the preparation of parenteral formulations are solvents, cosolvents and liquid or semisolid carriers, such as sterile water, ethanol, glycerol, propylene glycol, polyethylene glycol, butanediol, fatty oils, short- and medium chain triglycerides, lecithin, polyoxyethylene castor oil derivatives; substances to adjust the osmolality and pH, such as sugars, especially glucose, sugar alcohols, especially mannitol, sodium chloride, sodium carbonate, citric acid, acetate, phosphate, phosphoric acid, hydrochloric acid, sodium hydroxide etc.; stabilizers, antioxidants, and preservatives, such as ascorbic acid, sodium sulfite or -hydrogen sulfite, EDTA, benzyl alcohol etc.; other excipients and lyophilization aids, such as albumin, dextran etc.

Alternatively, the pharmaceutical compositions may be designed for oral administration and processed accordingly. Appropriate oral dosage forms include tablets, hard capsules, soft capsules, powders, granules, orally disintegrating dosage forms, syrups, drops, suspensions, effervescent tablets,

chewable tablets, oral films, lyophilized dosage forms, sustained release dosage forms, controlled release dosage forms. In one of the preferred embodiments, the oral dosage form is an enterically coated solid dosage form to provide protection of the compound from the acidic and proteolytic environment of the stomach.

It may also be advantageous to administer a compound of the invention in a transmucosal dosage form. This route of administration is non-invasive and patient-friendly; at the same time it may lead to an improved bioavailability of the compound compared to oral administration, especially if the compound is not stable in the fluids of the digestive system, or if it is too large to be absorbed from the gut effectively. Transmucosal administration is possible via, for instance, nasal, buccal, sublingual, gingival, or vaginal dosage forms. These dosage forms can be prepared by known techniques; they can be formulated to represent nasal drops or sprays, inserts, films, patches, gels, ointments, or tablets. Preferably, the excipients used for a transmucosal dosage form include one or more substances providing for mucoadhesion, thus prolonging the contact time of the dosage form with the site of absorption and thereby potentially increasing the extent of absorption.

In a further embodiment, the compounds are administered via the pulmonary route, using a metered dose inhaler, a nebulizer, an aerosol spray, or a dry powder inhaler. Appropriate formulations can be prepared by known methods and techniques. Transdermal, rectal, or ocular administration may also be feasible in some cases.

It can be advantageous to use advanced drug delivery or targeting methods to deliver a compound of the invention more effectively. For instance, if a non-parenteral route of administration is chosen, an appropriate dosage form may contain a bioavailability enhancing agent, which may be any substance or mixture of substances which increases the availability of the compound. This may be achieved, for instance, by the protection of the compound from degradation, such as by an enzyme inhibitor or an antioxidant.

More preferably, the enhancing agent increases the bioavailability of the compound by increasing the permeability of the absorption barrier, which is typically a mucosa. Permeation enhancers can act via various mechanisms; some increase the fluidity of mucosal membranes, while others open or widen the gap junctions between mucosal cells. Still others reduce the viscosity of the mucus covering the mucosal cell layer. Among the preferred bioavailability enhancers are amphiphilic substances such as cholic acid derivatives, phospholipids, ethanol, fatty acids, oleic acid, fatty acid derivatives, EDTA, carbomers, polycarbophil, and chitosan.

In a further aspect, molecules capable of binding to the compounds disclosed above are within the scope of the invention. For instance, standard hybridyzation technology can be applied to prepare specific monoclonal antibodies to a compound. Other techniques are available to design and prepare smaller molecules capable of binding to a compound of the invention.

The following examples are intended to further illustrate the invention, but not to limit its scope to the embodiments presented therein.

Example 1:

a) Chemical synthesis of resin-bound core sequence 9 (Fig.1)

Core sequence 9 FmocHN-Glu(OtBu)-Trp(Boc)-Val-Asp(OtBu)-Val-Lys(DDE)-GABA-HMPA-resin was synthesized on an Applied Biosystems 9050 peptide synthesizer (Warrington, UK) using standard Fmoc chemistry. In short, Tentagel S-NH_2 (load 0.26 mmol/g) was provided with 4-hydroxymethylphenoxyacetic acid (HMPA) as a linker. Fmoc-GABA –OH (10 eq.) was attached to the HMPA resin under the agency of N,N'-dicyclohexylcarbodiimide (DCC, 5 eq.) and 4-dimethylaminopyridine (DMAP, 0.5 eq.) All other amino acids, with acid labile side chain protection if necessary, were attached by coupling in the presence of HOBt/TBTU/Dipea.

After coupling, the resin was washed with DMF, isopropanol and diethylether and subsequently dried.

b) Synthesis of compound 10 (Fig.1)

The solid phase synthesis of the compound library of Fig. 1 was performed using a Flexchem© system (Robbins Scientific, Sunnyvale U.S.A.). After removal of the N-terminal Fmoc group of 9 by 20% piperidine in DMF, the resin was washed (DMF) and dried. The resin was distributed in 10 mg quantities over a solvent-resistant 48-well filter plate. After washing with DMF, a mixture of the desired carboxylic acid (40 eq.), BOP (20 eq.) and NMM (100 eq.) was added (total volume 300 µl) and the suspended resin was incubated for 3 hours under continuous perspiration with N_2. Subsequently, the resin was washed with DMF and incubated three times for 3 minutes with hydrazine monohydrate (2% in DMF) to remove the DDE group. After washing with DMF, a mixture of the second carboxylic acid, BOP, HOBt and NMM (same amounts as described above) was added and once again incubated for 3 hours. Peptides, which were only modified at the C-terminal lysine, were N-boculated with di-tert.-butyl-dicarbonate ($(t\text{-}Boc)_2O$) and Dipea prior to reaction to protect the N-terminal amine function. After removal of the solvent, peptides were cleaved off from the resin with a mixture of trifluoro acetic acid (TFA), triisopropylsilane, water (95:2.5:2.5 v/v/v). Each sample was lyophilized and stored at -20°C until use.

In a corresponding manner, compounds of formula 10 (Fig.1) were prepared. In these compounds, Z is -CO-, and R^1 and/or R^2 represent independently from each other the following groups:

4-hydroxyphenylcarbonyl;

3,5-dihydroxyphenylcarbonyl;

3-hydroxy-5-carbonyloxyphenylcarbonyl

3,4,5-tri-methoxy-phenylcarbonyl

3,5-di-nitro-phenylcarbonyl

4-carboxyphenylcarbonyl

3-carbonylbutyric acid
2-hydroxy-5-sulfonylphenylcarbonyl
3-carboxyphenylcarbonyl
3,5-difluorophenylcarbonyl
1-sulfonyl-2-aminoethylcarbonyl
3,5-di(trifluoromethyl)phenylcarbonyl
hydroxymethylcarbonyl
4-nitrophenylcarbonyl

Example 2:

Performance of test and test results:

The affinity of the compounds to P-selectin was investigated by using an adapted ELISA method. Streptavidin-horseradish peroxidase (strepPO) was incubated with TM11-biotin in a 1 to 4 molar ratio for 2 hours at RT, hereby forming a tetrameric peptide strepPO-complex. For competition studies, microtiter wells were coated with chimeric human P-selectin as described for the isolation of P-selectin binding phage. Wells were then incubated with 2.5 nM TM11-strepPO complex in assay buffer for 1 hour at 4°C, in the presence of titered amounts of peptides to compete for binding to human P-selectin. Linear compounds were tested in the presence of DTT to prevent aggregate formation. After washing 6 times with assay buffer, the wells were incubated with 100 µl TMB/H_2O_2 for 15 min at RT. The reaction was stopped with 1 M H_2SO_4 and the absorbance read at 450 nm. From the results, the affinity constants were calculated:

Table 1:

Peptide	Affinity/ EC_{50} (μM)
HP 14	1.02
HP 16	0.31
HP 17	0.45
HP 61	0.19
HP 62	0.28
HP 63	3.40
HP 65	2.35
HP 60	1.10
HP 06	6.00

The peptides are coded HPij, wherein i and j refer to the acyl moieties R^1 and R^2 attached to the N- and C-terminus, respectively, of formula 10 (Fig.1). Unmodified amino group is indicated by 0. The acyl moieties 1 to 7 are shown in Fig. 2.

The affinity of the compounds was confirmed by a cell adhesion assay in which Chinese hamster ovary cells expressing human P-selectin were incubated with HL60 cells expressing PSGL-1 in the presence of titered amounts of the compounds.

Claims

1. A compound with affinity to human P-selectin, comprising a peptide with an amino acid sequence $X(A_x)_mA_3A_1A_2A_1Y$, or a functional equivalent of said peptide, wherein

A_1 is a D- or L-cysteine (C), or a D- or L-valine (V), or an analogue thereof;

A_2 is D- or L-aspartic acid (D) or an analogue thereof;

A_3 is D- or L-phenylalanine (F), or a D- or L-tryptophan (W), or an analogue thereof;

A_x is D- or L-amino acid, selected from the group consisting of glutamic acid (E), aspartic acid (D), glycine (G) and cysteine (C);

X marks the N-terminal side of said sequence and is hydrogen or a residue comprising 1 to 6 D- or L-amino acids or analogues thereof;

Y marks the C-terminal side of said sequence and is -OH or a residue comprising 1 to 11 D- or L-amino acids or analogues thereof;

wherein X and Y together may form a cyclic system;

wherein at least one of X and Y or X+Y is substituted with the group $R^1-(Z)_n-$;

wherein Z is selected from -CO-, -O-, $-NR^2-$, and $-CO-NR^2-$;

wherein R^1 and R^2 are independently selected from:

a) H;

b) a C_1-C_8 alkyl group;

c) a C_2-C_8 alkyl group, wherein at least one C-atom is replaced with a nitrogen-, oxygen- or sulphur atom;

d) a C_6-C_{14} aryl group, which may be substituted with at least one group selected from a halogen, C_1-C_6-alkyl, $-CF_3$, -OH, -O-C_1-C_6-alkyl, -COOH, -COO-C_1-C_6-alkyl, $-NO_2$, $-NH_2$, -NH-C_1-C_6-alkyl, -N-(C_1-C_6-alkyl)$_2$ and $-SO_3H$;

e) a heteroaryl group which is selected from 5- or 6-membered ring systems and benzo-condensed ring systems, and has at least one heteroatom

selected from the group consisting of nitrogen, oxygen and sulphur, wherein said heteroaryl group may be substituted with at least one group selected from the group consisting of a halogen, -C_1-C_6-alkyl, -CF_3, -OH, -O-C_1-C_6-alkyl, -COOH, -COO-C_1-C_6-alkyl, -NO_2, -NH_2, -NH-C_1-C_6-alkyl, -N-(C_1-C_6-alkyl)$_2$ and -SO_3H;

f) an aralkyl group comprising an alkyl group as defined in b) or c) and an aryl group or heteroaryl group as defined in d) or e);

and wherein m and n are integers independently selected from 0 and 1, with the proviso that n is not 0 when R^1 is H.

2. The compound according to claim 1, wherein A_x represents D- or L-glutamic acid (E) or D- or L- aspartic acid.

3. The compound according to claim 1 or 2, wherein A_1 represents D- or L-valine (V).

4. The compound according to any one of the preceding claims, wherein A_3 is D- or L-tryptophan (W).

5. The compound according to any one of the preceding claims, wherein Y is a residue comprising D- or L- lysine.

6. The compound according to any one of the preceding claims, wherein R^1 is unsubstituted phenyl or phenyl substituted with at least one substituent as defined in claim 1.

7. The compound according to any one of the preceding claims, wherein n is 0 and R^1 is 3,4,5-trihydroxyphenyl or 3,5-dicarboxyphenyl.

8. The compound according to any one of the preceding claims, wherein X comprises no amino acids and Y comprises D- or L-lysine.

9. The compound as claimed in claim 8, wherein n is 0 and R^1 is 3,4,5-trihydroxyphenyl or 3,5-dicarboxyphenyl.

10. The compound of claim 1, wherein m is 0, wherein Z is -CO-, and wherein Z is attached to Y via a D- or L-glycine or aminobutyric acid spacer.

11. The compound according to any one of the preceding claims, comprising a cyclic or constrained backbone structure.

12. The compound according to any one of the preceding claims, comprising at least two of said peptides or functional equivalents thereof, said compound comprising an affinity constant for binding to P-selectin which is lower than 1/20.

13. A method for the preparation of a compound according to any one of any one of the preceding claims, comprising a sequence of steps wherein amino acid monomers, amino acid oligomers, or mono- or oligomers of amino acid analogues or mimetics are assembled by chemical or enzymatic ligation, and wherein said steps are performed in a liquid phase and/or at the interface to a functionalized solid phase.

14. The method according to claim 13, comprising reacting the HMPA linker of the formula 8 (Fig.1) by standard Fmoc chemistry to yield a compound of the sequence $X(A_x)_mA_3A_1A_2A_1Y$, wherein X, A_x, A_3, A_1, A_2, Y and m are as defined in claim 1, and wherein the amino groups are initially protected by protecting groups, and R-CO is introduced by replacing the protecting groups by using standard methods.

15. A method for the preparation of a compound according to any one of claims 1 to 12, comprising the steps of:

(a) combining a nucleic acid sequence encoding the compound or a peptide similar to the compound with a vector capable of transfecting cells, such as a viral vector, a lipid vector, or a polymeric vector;

(b) transfecting host cells with the vector and the nucleic acid sequence associated therewith;

(c) culturing said host cells under conditions which allow the expression of the compound or the peptide similar to the compound;

(d) isolating the compound or the peptide similar to the compound; and, optionally,

(e) modifying the peptide similar to the compound to obtain the compound.

16. A method for the preparation of a compound according to any one of claims 1 to 12, comprising the steps of:

(a) combining a nucleic acid sequence encoding the compound or a peptide similar to the compound with a vector capable of transfecting cells, such as a viral vector, a lipid vector, or a polymeric vector;

(b) transfecting host cells with the vector and the nucleic acid sequence associated therewith, said host cells being parts of a living human organism.

17. A method for the preparation of a compound according to any one of claims 1 to 12, comprising the steps of:

(a) combining a nucleic acid sequence encoding the compound with a vector capable of transfecting cells, such as a viral vector, a lipid vector, or a polymeric vector;

(b) transfecting cells within a living human organism with the vector and the nucleic acid sequence associated therewith.

18. Use of a compound according to any one of claims 1 to 12 as a medicine or diagnostic.

19. Use of a compound according to any one of claims 1 to 12 for the manufacture of a medicament for inhibiting leukocyte binding to platelets and/or endothelial cells.

20. The use according to claim 18 or 19 for the manufacture of a medicament for the treatment, prevention, or diagnosis of chronic inflammatory disorders, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, atherosclerosis, restenosis, ischemia, reperfusion injury including renal failure, tumor metastasis, bacterial sepsis, disseminated intravascular coagulation, adult respiratory stress syndrome, stroke, angiogenesis, transplant rejection, thrombosis, or circulatory shock.

21. Pharmaceutical composition, comprising a compound according to any one of claims 1 to 12 and one or more pharmaceutically acceptable carriers or excipients.

22. Pharmaceutical composition according to claim 21, which is formulated and processed for parenteral administration, preferably for intravascular, intramuscular, subcutaneous, or intralesional injection.

23. Pharmaceutical composition according to claim 21, which is formulated and processed for oral administration, preferably in form of a tablet, a capsule, granules, an enteric solid dosage form, a solid dosage form

providing sustained or controlled release, or an orally disintegrating dosage form.

24. Pharmaceutical composition according to claim 21, which is formulated and processed for transmucosal administration, such as nasal, buccal, sublingual, or vaginal administration.

25. Pharmaceutical composition according to claim 21, which is formulated and processed for pulmonary administration through a metered dose inhaler, a nebulizer, an aerosol spray dispenser, or a dry powder inhaler.

26. Pharmaceutical composition according to any one of claims 21 to 25, further comprising a drug targeting agent and/or a bioavailability enhancing agent.

27. A method for determining whether a molecule comprises a binding affinity for P-selectin comprising contacting P-selectin or a functional equivalent thereof with said molecule and with a compound according to any one of claims 1-12 and determining whether binding of said compound to said P-selectin or functional analogue thereof, is reduced.

28. A binding molecule capable of specifically binding a compound according to any one of claims 1-12.

29. A binding molecule according to claim 28, comprising an antibody or a functional part, derivative and/or analogue thereof.

30. A method for determining whether a compound is capable of binding to human P-selectin, comprising substituting in a compound according to any one of claims 1-12, an amino acid for a conservative amino acid and

determining whether the resulting compound is capable of binding to said P-selectin.

Title: Compounds binding to P-selectin

Abstract

The present invention relates to compounds which bind selectively to the adhesion molecule human P-selectin, and particularly to such compounds comprising a peptide with an amino acid sequence $X(A_x)_nA_3A_1A_2A_1Y$, or a functional equivalent of said peptide. In addition, the invention relates to methods for preparing such compounds, to the use of such compounds in therapeutic or diagnostic methods and in pharmaceutical compositions, to nucleic acids encoding for proteinaceous materials comprising the amino acid sequences of said compounds, to gene delivery vehicles comprising such nucleic acids, to binding molecules binding to said compounds, and to a method for determining whether a compound is capable of binding to P-selectin.

Figure 1

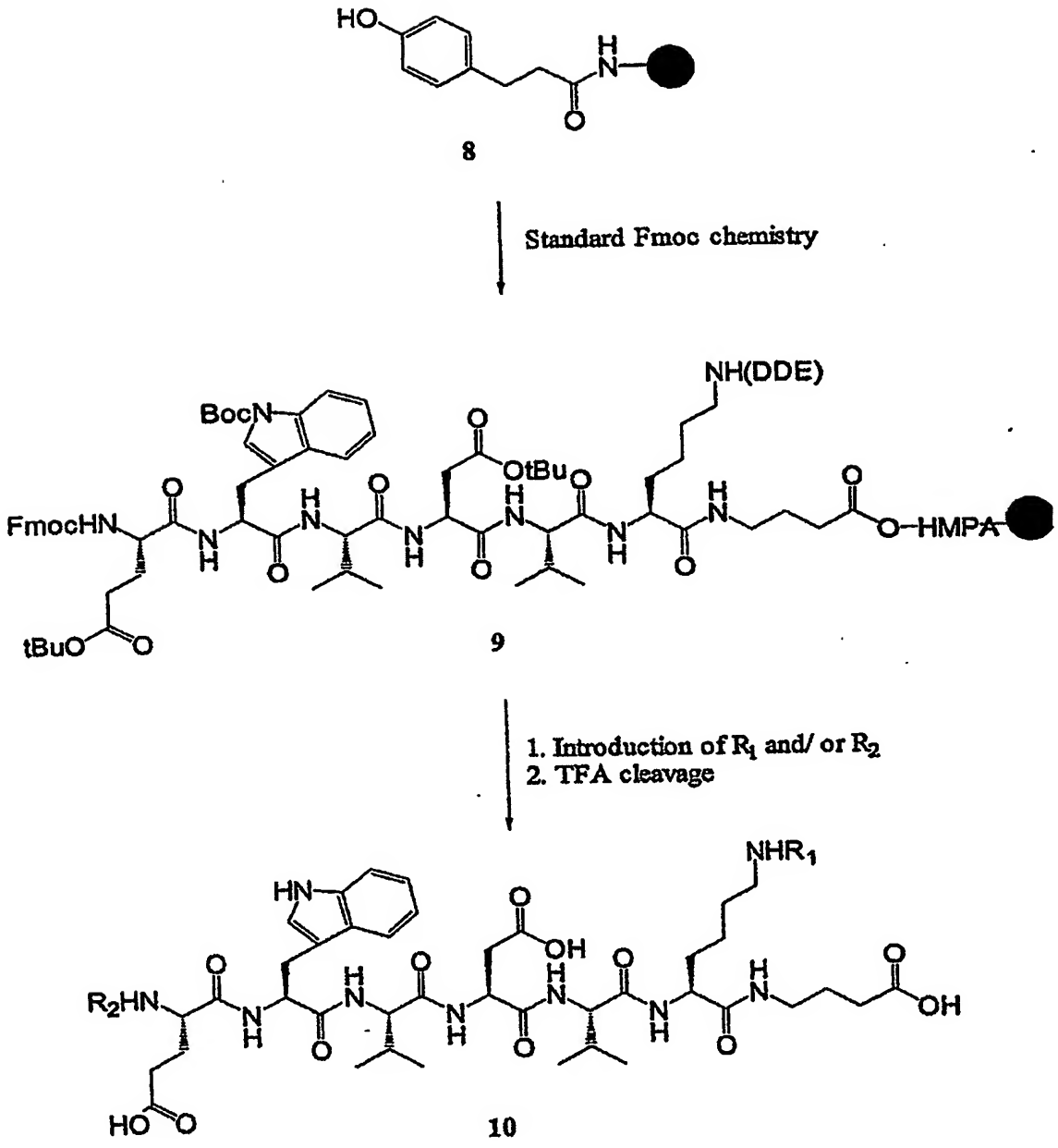

Figure 2